

DESCRIPTION

PEPTIDOMIMETIC INHIBITORS OF STAT ACTIVITY AND USES THEREOF

5 This invention was made with government support under National Cancer Institute grants CA78038 and CA55652. The government has certain rights in the invention.

Cross-Reference to Related Application

10 The application claims priority to U.S. Provisional Application Serial Number 60/319,960, filed February 20, 2003, which is hereby incorporated by reference in its entirety, including all figures, nucleic acid sequences, amino acid sequences, and tables.

Field of Invention

15 This invention relates to materials and methods for blocking tumor growth and inducing tumor cell death by disrupting the activity of a STAT transcription factor protein, such as Stat3.

Background of the Invention

20 Knowledge of the molecular basis of cancer potentially expands the number of strategies to target cancer cells for therapy. Multiple genetic alterations in cancer frequently result in aberrations in the biochemical properties of signaling molecules, leading to dysregulation of signal transduction mechanisms in tumors and consequently malignant progression. As distinct molecular features of tumors compared to normal cells, these molecular changes conceptually provide unique targets for the design of tumor-selective drugs.

25 Signal Transducer and Activator of Transcription (STAT) proteins are latent cytoplasmic transcription factors that are activated in response to cytokines and growth factors and consequently regulate cellular processes, including proliferation, differentiation and survival (Darnell, J.E., Jr. (2002); Horvath, C.M. (2000); Darnell, J.E., Jr. (1997); Schindler, C. and Darnell, J.E., Jr. (1995); Bromberg, J. and Darnell, J.E., Jr. (2000); Stark, G.R. *et al.* (1998); Smithgall, T.E. *et al.* (2000); Akira, S. (2000); Hirano, T. *et al.* (2000); Kotenko, S.V. and
30 Pestka, S. (2000)). STAT activation is dependent on tyrosine phosphorylation, which induces

dimerization via reciprocal phosphotyrosine (pTyr)-SH2 interactions between two STAT monomers and is required for binding to specific DNA response elements (Shuai, K. *et al.* (1993); Shuai, K. *et al.* (1994); Sasse, J. *et al.* (1997)).

A large number of studies on persistent activation of specific STAT family members, particularly Stat3, have established a strong link to growth and survival of transformed and tumor cells (Bowman, T. *et al.* (2000a); Catlett-Falcone, R. *et al.* (1999a); Garcia, R. and Jove, R. (1998); Turkson, J. and Jove, R. (2000); Song, J.I. and Grandis, J.R. (2000); Lin, T.S. *et al.* (2000)). In a number of human solid and hematological tumors, studies have identified a high frequency of abnormal activation of Stat3. In many tumor cells harboring persistent Stat3 activity, inhibition of Stat3 signaling induces growth arrest and apoptosis. The critical role of Stat3 in the molecular pathogenesis of many diverse tumors provides validation for its targeting in cancer drug discovery (Turkson, J. and Jove, R. (2000); Buettner, R. *et al.* (2002)).

Brief Summary of the Invention

The subject invention concerns compositions and methods for blocking cancer cell growth or proliferation and/or inducing cancer cell death. Compositions of the present invention are peptidomimetics that inhibit STAT function. Peptidomimetics of the invention include compounds of the formula RY*L (where Y* represents phosphotyrosine), with the R group at the Y-1 position. Peptidomimetics of the invention disrupt Stat3 activation and function. Consistent with their activity of inhibiting Stat3, representative peptidomimetics of the invention significantly inhibit tumor cell growth and induce tumor cell death.

Brief Description of the Drawings

Figures 1A and 1B show electrophoretic mobility shift assay (EMSA) analyses of STAT DNA-binding activities showing effects of peptidomimetics. Nuclear extracts containing activated Stat1, Stat3 and Stat5 are treated with the indicated concentrations of peptidomimetics designated ISS 493, ISS 610, ISS 637, ISS 593, ISS 221, or ISS 610NP for 30 min at room temperature prior to incubation with radiolabeled oligonucleotide probes. Figure 1A shows Stat1 and Stat3 binding to hSIE probe (SEQ ID NO. 3), and Figure 1B shows Stat1 and Stat5 binding to MGFe probe (SEQ ID NO. 4). Positions of STAT:DNA complexes in gel are labeled. Control

lanes represent nuclear extracts from NIH3T3 cells stimulated with EGF but not treated with peptidomimetics.

Figures 2A and 2B show evidence for dissociation of STAT dimers by phosphopeptide or peptidomimetic. Cell lysates contain either only activated Stat1 (lane 1), Stat3 (lane 2) or both (pooled lysates, shown in lanes 3-10) and are treated (lanes 4-10) with the indicated concentrations of PY*LKTK (SEQ ID NO. 5) (Figure 2A) or ISS 610 (Figure 2B) for 30 min at room temperature prior to incubation with radiolabeled hSIE oligonucleotide probe (SEQ ID NO. 3). Positions of STAT:DNA complexes in gel are labeled. Cell lysates were prepared from recombinant baculovirus-infected Sf-9 cells as described in the Materials and Methods section.

Figures 3A-3F show evaluation of peptidomimetic effects on Stat3 activation and Stat3-mediated gene expression in intact cells, and on Src-transformation. Figures 3A and 3B show luciferase activities in extracts prepared from peptidomimetic designated ISS 610- or ISS 610NP-treated v-Src-transformed mouse fibroblasts that stably express Stat3-dependent (NIH3T3/v-Src/pLucTKS3) and Stat3-independent (NIH3T3/v-Src/pRLSRE) luciferase reporters. Values are the means and S.D. of three independent assays. Figures 3C-3E show EMSA analyses of Stat3 DNA-binding activities (using hSIE oligonucleotide probe) (SEQ ID NO. 3) in nuclear extracts prepared from v-Src-transformed NIH3T3/v-Src (Figures 3C and 3D), human breast carcinoma MDA-MB-435, MDA-MB-468, and MDA-MB-231 (Figure 3E). Figure 3F shows the effect of ISS 610 peptidomimetic on soft-agar growth of v-Src-transformed fibroblasts (NIH3T3/v-Src) and their v-Ras-transformed counterparts (NIH3T3/v-Ras). Transformed cells were seeded in soft agar and treated every 2-3 days with or without 1mM ISS 610 peptidomimetic until large colonies were evident. Values are the mean and S.D. of the three independent assays.

Figures 4A-C show evaluation of peptidomimetic effects on cell proliferation. Growth curves for transformed and tumor cells. Normal and transformed fibroblasts (NIH3T3, NIH3T3/v-Src, or NIH3T3/v-Ras) as well as human breast carcinoma (MDA-MB-231, MDA-MB-435, or MDA-MB-453) cells were treated with or without compounds and counted by trypan blue exclusion on each of four days. Cells were untreated (dotted lines) or treated with 1 mM ISS 610 or PY*LKTK-MTS (SEQ ID NO. 1) (solid lines). Values are the mean and S.D. of four independent determinations.

Figure 5 shows computer modeling of peptidomimetic ISS 610 bound to SH2 pocket. Comparison of the lowest energy GOLD (Jones *et al.* (1997)) docked conformation of ISS 610 (green), and the C-terminal phosphotyrosine peptide, AAPY*LK (SEQ ID NO. 6), of the associated Stat3 β monomer as determined from the crystal structure (orange), in the SH2 binding domain of Stat3 β (pale blue) (Becker, S., *et al.* (1998)).

Brief Description of the Sequences

SEQ ID NO. 1 is a peptide comprising a membrane translocating sequence.

SEQ ID NO. 2 is a peptide comprising a membrane translocating sequence.

SEQ ID NO. 3 is an oligonucleotide probe designated as hSIE that binds to STAT proteins.

SEQ ID NO. 4 is an oligonucleotide probe designated as MGFe that binds to STAT proteins.

SEQ ID NO. 5 is a peptide.

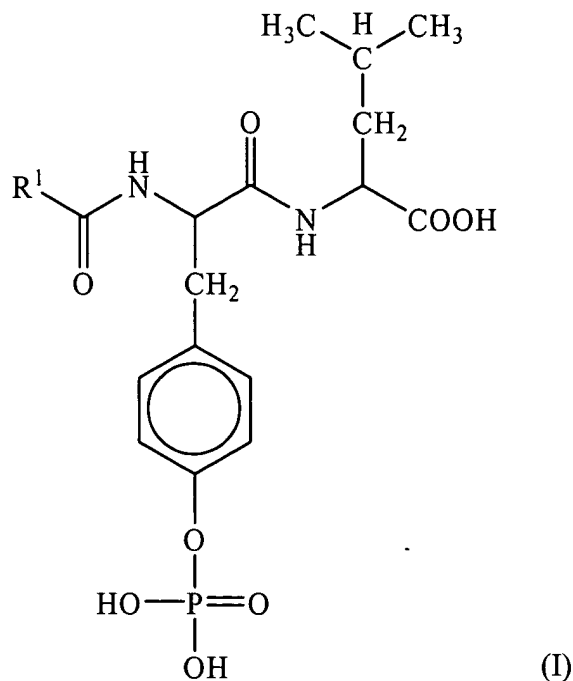
SEQ ID NO. 6 is a peptide.

Detailed Disclosure of the Invention

The subject invention concerns compositions for blocking cancer cell growth or proliferation and/or inducing cancer cell death. Compositions of the invention comprise peptidomimetic molecules that are inhibitors of STAT proteins. Peptidomimetics within the scope of the invention include peptidomimetics having the structure R-Y*L, where R is bound via the Y-1 position and is an organic group as defined herein and Y* is a phosphotyrosine.

In one embodiment, a peptidomimetic of the invention has the structure shown in formula I:

I:



(I)

wherein

R¹ is alkyl, alkoxy, cycloalkyl, cycloalkoxy, aryl, aryloxy, alkylcarbonyl, alkoxycarbonyl, cycloalkylcarbonyl, heteroalkyl, heterocycloalkyl, heterocycloalkylcarbonyl, heteroaryl, arylcarbonyl, heteroarylcarbonyl, aryloxycarbonyl, heteroaryloxycarbonyl, heterocycloalkoxy, or heterocycloalkoxycarbonyl, any of which can be optionally substituted with one or more of the following: any halogen, -CN, -COOH, =O, -OH, -NO₂, -NH₂, -N-alkyl, alkyl including -CH₃, alkoxy including -OCH₃, cycloalkyl, cycloalkoxy, aryl, aryloxy, alkylcarbonyl, alkoxycarbonyl, cycloalkylcarbonyl, heteroalkyl, heterocycloalkyl, heterocycloalkylcarbonyl, heteroaryl, arylcarbonyl, heteroarylcarbonyl, aryloxycarbonyl, heteroaryloxycarbonyl, heterocycloalkoxy, or heterocycloalkoxycarbonyl;

or a salt thereof.

In a preferred embodiment, peptidomimetics of the invention have an R¹ group selected from phenyl, pyridyl, and pyrazinyl moieties. In one embodiment, R¹ is phenyl optionally substituted with one or more halogen, -NO₂, -CN or -OCH₃. In an exemplified embodiment, R¹ is phenyl substituted with -OCH₃ or -CN. In another embodiment, R¹ is pyridyl optionally

substituted with one or more halogen or -CH₃. In an exemplified embodiment, R¹ is pyridyl substituted with Cl and F.

As used herein, alkyl means straight or branched chain, saturated or mono- or polyunsaturated hydrocarbon groups having from 1 to 20 carbon atoms and C_{1-X} alkyl means
5 straight or branched chain alkyl groups containing from one up to X carbon atoms wherein X is any positive integer. For example, C₁₋₆ alkyl means straight or branched chain alkyl groups containing from one up to 6 carbon atoms. Alkoxy means an alkyl-O-group in which the alkyl group is as previously described. Cycloalkyl includes a nonaromatic monocyclic or multicyclic ring system, including fused and spiro rings, of from about three to about 10 carbon atoms. A
10 cyclic alkyl may optionally be partially unsaturated. Cycloalkoxy means a cycloalkyl-O-group in which cycloalkyl is as defined herein. Aryl means an aromatic monocyclic or multicyclic carbocyclic ring system, including fused and spiro rings, containing from about six to about 14 carbon atoms. Aryloxy means an aryl-O-group in which the aryl group is as described herein. Alkylcarbonyl means a RC(O)- group where R is an alkyl group as previously described.
15 Alkoxy carbonyl means an ROC(O)- group where R is an alkyl group as previously described. Cycloalkylcarbonyl means an RC(O)- group where R is a cycloalkyl group as previously described. Cycloalkoxy carbonyl means an ROC(O)- group where R is a cycloalkyl group as previously described.

Heteroalkyl means a straight or branched-chain having from one to 20 carbon atoms and
20 one or more heteroatoms selected from nitrogen, oxygen, or sulphur, wherein the nitrogen and sulphur atoms may optionally be oxidized, *i.e.*, in the form of an N-oxide or an S-oxide. Heterocycloalkyl means a monocyclic or multicyclic ring system (which may be saturated or partially unsaturated), including fused and spiro rings, of about five to about 10 elements wherein one or more of the elements in the ring system is an element other than carbon and is
25 selected from nitrogen, oxygen, silicon, or sulphur atoms. Heteroaryl means a five to about a 14-membered aromatic monocyclic or multicyclic hydrocarbon ring system, including fused and spiro rings, in which one or more of the elements in the ring system is an element other than carbon and is selected from nitrogen, oxygen, silicon, or sulphur and wherein an N atom may be in the form of an N-oxide. Arylcarbonyl means an aryl-CO-group in which the aryl group is as
30 described herein. Heteroarylcarbonyl means a heteroaryl-CO-group in which the heteroaryl

group is as described herein and heterocycloalkylcarbonyl means a heterocycloalkyl-CO-group in which the heterocycloalkyl group is as described herein. Aryloxycarbonyl means an ROC(O)-group where R is an aryl group as previously described. Heteroaryloxycarbonyl means an ROC(O)- group where R is a heteroaryl group as previously described. Heterocycloalkoxy means a heterocycloalkyl-O- group in which the heterocycloalkyl group is as previously described. Heterocycloalkoxycarbonyl means an ROC(O)- group where R is a heterocycloalkyl group as previously described.

Examples of saturated alkyl groups include, but are not limited to, methyl, ethyl, N-propyl, isopropyl, N-butyl, tert-butyl, isobutyl, sec-butyl, N-pentyl, N-hexyl, N-heptyl, and N-octyl. An unsaturated alkyl group is one having one or more double or triple bonds. Unsaturated alkyl groups include, for example, ethenyl, propenyl, butenyl, hexenyl, vinyl, 2-propynyl, 2-isopentenyl, 2-butadienyl, ethynyl, 1-propynyl, 3-propynyl, and 3-butylnyl. Cycloalkyl groups include, for example, cyclopentyl, cyclohexyl, 1-cyclohexenyl, 3-cyclohexenyl, and cycloheptyl. Heterocycloalkyl groups include, for example, 1-piperidiny, 2-piperidiny, 3-piperidiny, 3-morpholinyl, 4-morpholinyl, tetrahydrofuran-2-yl, tetrahydrofuran-3-yl, tetrahydrothien-2-yl, tetrahydrothien-3-yl, 1-piperazinyl, 2-piperazinyl, and 1,4-diazabicyclooctane. Aryl groups include, for example, benzyl, phenyl, indenyl, biphenyl, 1-naphthyl, 2-naphthyl, anthracenyl, and phenanthracenyl. Heteroaryl groups include, for example, 1-pyrrolyl, 2-pyrrolyl, 3-pyrrolyl, furyl, thienyl, imidazolyl, oxazolyl, isoxazolyl, thiazolyl, pyrazolyl, pyridyl, pyrimidyl, purinyl, indolyl, quinolinyl, isoquinolinyl, benzoquinolinyl, carbazolyl, and diazaphenanthrenyl.

As used herein, halogen means the elements fluorine (F), chlorine (Cl), bromine (Br), and iodine (I).

The subject invention also concerns compositions comprising a peptidomimetic of the invention, or a salt thereof, in a pharmaceutically acceptable carrier or diluent.

Examples of peptidomimetics of the invention are shown in Table 3 and have been designated with an "ISS" number. Peptidomimetics of the invention, such as those exemplified herein and designated as ISS 221, ISS 437, ISS 593, ISS 610 and ISS 637 are potent disrupters of active Stat3 and effectively disrupt and dissociate active Stat3:Stat3 dimers. Shown in Figure 5 is the lowest energy GOLD docked conformation of peptidomimetics ISS 610 in the SH2 domain of Stat3 β , as compared to the observed SH2 domain-bound phosphopeptide AAPY*LK (SEQ ID

NO. 6) of the associated monomer of Stat3 β in the X-ray crystal structure (Becker, S., *et al.* (1998). The modeling by GOLD flexible docking program reveals that ISS 610 has access to the hydrophobic pocket and available hydrogen bonding interactions on the protein surface. R substituents of formula I include 4-cyanobenzoate (ISS 610), 2,6-dimethoxybenzoate (ISS 637),
5 2-methyl pyridine-3-carboxylic acid (ISS 221), 2,6-dichloro-4-fluoro pyridine-3-carboxylic acid (ISS 593) as well as 5-methylpyrazine carboxylic acid (ISS 493). While peptidomimetics with pyrazinyl or phenyl substitutions are generally more selective for Stat3, the selectivity of these peptidomimetics may be influenced by the presence and the type, as well as the positioning, of functional groups on the aromatic ring.

10 Salts of the peptidomimetics of the invention include those which are prepared with acids or bases, depending on the particular substituents present on the subject peptidomimetics described herein. Examples of a base addition salts include sodium, potassium, calcium, ammonium, or magnesium salt. Examples of acid addition salts include hydrochloric, hydrobromic, nitric, phosphoric, carbonic, sulphuric, and organic acids like acetic, propionic,
15 benzoic, succinic, fumaric, mandelic, oxalic, citric, tartaric, maleic, and the like. Salts of platinum complexes of the invention can be prepared using conventional techniques.

Peptides of the invention can be readily prepared using standard techniques known in the art, including chemical synthesis (Merrifield, 1963) and genetic engineering. Peptidomimetics of the invention can be synthesized or prepared from peptides using standard chemical procedures
20 and materials.

Peptidomimetics having substitution of amino acids other than those specifically exemplified in the subject peptidomimetics are also contemplated within the scope of the present invention. For example, non-natural amino acids can be substituted for the amino acids of a peptidomimetic of the invention, so long as the peptidomimetic having substituted amino acid(s)
25 retains substantially the same activity as the peptidomimetic in which amino acid(s) have not been substituted. Examples of non-natural amino acids include, but are not limited to, ornithine, citrulline, hydroxyproline, homoserine, phenylglycine, taurine, iodotyrosine, 2,4-diaminobutyric acid, α -amino isobutyric acid, 4-aminobutyric acid, 2-amino butyric acid, γ -amino butyric acid, ϵ -amino hexanoic acid, 6-amino hexanoic acid, 2-amino isobutyric acid, 3-amino propionic acid,
30 norleucine, norvaline, sarcosine, homocitrulline, cysteic acid, τ -butylglycine, τ -butylalanine,

phenylglycine, cyclohexylalanine, β -alanine, fluoro-amino acids, designer amino acids such as β -methyl amino acids, C-methyl amino acids, N-methyl amino acids, and amino acid analogues in general. Non-natural amino acids also include amino acids having derivatized side groups. Furthermore, any of the amino acids in the protein can be of the D (dextrorotary) form or L (levorotary) form.

Amino acids can be generally categorized in the following classes: non-polar, uncharged polar, basic, and acidic. Table 1 below provides a listing of examples of amino acids belonging to each class.

Table 1.	
Class of Amino Acid	Examples of Amino Acids
Nonpolar	Ala, Val, Leu, Ile, Pro, Met, Phe, Trp
Uncharged Polar	Gly, Ser, Thr, Cys, Tyr, Asn, Gln
Acidic	Asp, Glu
Basic	Lys, Arg, His

Conservative substitutions whereby a peptidomimetic having an amino acid of one class is replaced with another amino acid of the same class fall within the scope of the subject invention so long as the peptidomimetic having the substitution still retains substantially the same biological activity as a peptidomimetic that does not have the substitution. Thus, peptidomimetics of the invention having the structure R^1Y^*L (wherein R^1 is as defined herein) include those where the leucine (L) residue is replaced with a nonpolar amino acid, such as valine, alanine, *etc.* Also specifically contemplated within the scope of the invention are compounds of formula R^1Y^*L having analogs of Y^* or L wherein the peptidomimetic having the analog has substantially the same biological activity as a non-analog peptidomimetic. For example, analogs of Y^* include those where the aromatic ring of phosphotyrosine can be substituted with various substituents including, but not limited to, any halogen, -OH, -NO₂, -NH₂, -COOH, alkyl (such as -CH₃), and alkoxy (such as -OCH₃).

Single letter amino acid abbreviations are defined in Table 2.

Table 2.	
Letter Symbol	Amino Acid
A	Alanine
B	Asparagine or aspartic acid
C	Cysteine
D	Aspartic Acid
E	Glutamic Acid
F	Phenylalanine
G	Glycine
H	Histidine
I	Isoleucine
K	Lysine
L	Leucine
M	Methionine
N	Asparagine
P	Proline
Q	Glutamine
R	Arginine
S	Serine
T	Threonine
V	Valine
W	Tryptophan
Y	Tyrosine
Z	Glutamine or glutamic acid

5 The subject invention also concerns methods for inhibiting the growth or replication of a cell having abnormal growth or replication or whose growth or replication is uncontrolled, such as a cancer cell. In one embodiment, methods of the invention comprise inhibiting function of a STAT by contacting a cell expressing a STAT with a peptidomimetic of the invention wherein the peptidomimetic is taken in or otherwise provided inside the cell. In one embodiment, the cell is a tumor cell, cancer cell, or a transformed cell. The cell can be a cell from a mammal, including human, dog, cat, and horse. The types of cells encompassed within the scope of the invention include, but are not limited to, cells of breast, kidney, mouth, larynx, esophagus,

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stomach, testis, cervix, head, neck, colon, ovary, lung, bladder, skin, muscle, pancreas, prostate, bone, eye, blood cells, and brain.

Methods of the invention also comprise inhibiting the function and/or growth and replication of a cell that is aberrantly or constitutively expressing a STAT, such as Stat3. In one embodiment, the method comprises contacting a cell with a peptidomimetic of the invention. In one embodiment, the cell is a tumor cell, cancer cell, or a transformed cell. The cell can be a cell from a mammal, including human, dog, cat, and horse. The types of cells encompassed within the scope of the invention include, but are not limited to, cells of breast, kidney, mouth, larynx, esophagus, stomach, testis, cervix, head, neck, colon, ovary, lung, bladder, skin, muscle, pancreas, prostate, bone, eye, blood cells, and brain.

The subject invention also concerns methods for inducing apoptosis in a target cell. In one embodiment, the method comprises contacting a cell with a peptidomimetic of the invention. In one embodiment, the cell is a tumor cell, cancer cell, or a transformed cell. The cell can be a cell from a mammal, including human, dog, cat, and horse. The types of cells encompassed within the scope of the invention include, but are not limited to, cells of breast, kidney, mouth, larynx, esophagus, stomach, testis, cervix, head, neck, colon, ovary, lung, bladder, skin, muscle, pancreas, prostate, bone, eye, blood cells, and brain.

Peptidomimetics of the invention can be delivered to a cell either through direct contact with the cell or via a carrier means. Carrier means for delivering compositions to cells are known in the art and include encapsulating the composition in a liposome moiety, and attaching the platinum complexes to a protein or nucleic acid that is targeted for delivery to the target cell. Published U.S. Patent Application Nos. 20030032594 and 20020120100 disclose amino acid sequences that can be coupled to a composition and that allows the composition to be translocated across biological membranes. Published U.S. Patent Application No. 20020035243 also describes compositions for transporting biological moieties across cell membranes for intracellular delivery.

The subject invention also concerns methods for treating tumors and oncological disorders in a patient. In one embodiment, an effective amount of a peptidomimetic of the present invention is administered to a patient having an oncological disorder and who is in need of treatment thereof. The patient can be a human or other mammal, such as a primate (monkey,

chimpanzee, ape, *etc.*), dog, cat, cow, pig, or horse, or other animals having an oncological disorder. Means for administering and formulating a peptidomimetic for administration to a patient are known in the art, examples of which are described herein. Oncological disorders that can be treated using the subject invention include cancer and/or tumors of the breast, kidney, mouth, larynx, esophagus, stomach, testis, cervix, head, neck, colon, ovary, lung, bladder, skin, muscle, pancreas, prostate, bone, eye, blood cells, and brain. The peptidomimetics of the invention can also be used to treat other disorders that are associated with aberrant or constitutive expression of a STAT, such as Stat3.

For the treatment of tumors and oncological disorders, the peptidomimetics of this invention can be administered to a patient in need of treatment alone, or in combination with other antitumor or anticancer substances and/or with radiation therapy and/or with surgical treatment to remove a tumor or cancerous tissue. These other substances or radiation treatments may be given at the same or different times as the peptidomimetics of this invention. For example, the peptidomimetics of the present invention can be used in combination with mitotic inhibitors such as taxol or vinblastine, alkylating agents such as cisplatin, cyclophosphamide or ifosfamide, antimetabolites such as 5-fluorouracil or hydroxyurea, DNA intercalators such as adriamycin or bleomycin, topoisomerase inhibitors such as etoposide or camptothecin, antiangiogenic agents such as angiostatin, antiestrogens such as tamoxifen, and/or other drugs or antibodies that inhibit cancer cells, such as, for example, GLEEVEC (Novartis) and HERCEPTIN (Genetech), respectively.

Therapeutic application of the subject peptidomimetics, and compositions containing them, can be accomplished by any suitable therapeutic method and technique presently or prospectively known to those skilled in the art. The peptidomimetics can be administered by any suitable route known in the art including, for example, topical, oral, nasal, rectal, parenteral, subcutaneous, intramuscular, or intravenous routes of administration. Administration of the peptidomimetics of the invention can be continuous or at distinct intervals as can be readily determined by a person skilled in the art. The dosage to be administered to a patient can vary depending on several factors, including age, weight, and sex of the patient, and the type and severity of the disease. The ordinarily skilled clinician can determine suitable dosages following evaluation of the patient.

Compounds useful in the subject invention can be formulated according to known methods for preparing pharmaceutically useful compositions. Formulations are described in detail in a number of sources which are well known and readily available to those skilled in the art. For example, *Remington's Pharmaceutical Science* by E.W. Martin describes formulations which can be used in connection with the subject invention. In general, the compositions of the subject invention will be formulated such that an effective amount of the bioactive peptidomimetic is combined with a suitable carrier in order to facilitate effective administration of the composition. The compositions used in the present methods can also be in a variety of forms. These include, for example, solid, semi-solid, and liquid dosage forms, such as tablets, pills, powders, liquid solutions or suspension, suppositories, injectable and infusible solutions, and sprays. The preferred form depends on the intended mode of administration and therapeutic application. The compositions also preferably include conventional pharmaceutically acceptable carriers and diluents which are known to those skilled in the art. Examples of carriers or diluents for use with the subject peptidomimetics include, but are not limited to, water, saline, oils including mineral oil, ethanol, dimethyl sulfoxide, gelatin, cyclodextrans, magnesium stearate, dextrose, cellulose, sugars, calcium carbonate, glycerol, alumina, starch, and equivalent carriers and diluents, or mixtures of any of these. Formulations of the peptidomimetics of the invention can also comprise suspension agents, protectants, lubricants, buffers, preservatives, and stabilizers. To provide for the administration of such dosages for the desired therapeutic treatment, pharmaceutical compositions of the invention will advantageously comprise between about 0.1% and 45%, and especially, 1 and 15% by weight of the total of one or more of the peptidomimetics based on the weight of the total composition including carrier or diluent.

The peptidomimetics and compositions of the subject invention can also be administered utilizing liposome technology, slow release capsules, implantable pumps, and biodegradable containers. These delivery methods can, advantageously, provide a uniform dosage over an extended period of time.

The subject peptidomimetics can also be modified by the addition of chemical groups, such as PEG (polyethylene glycol). PEGylated peptides typically generate less of an immunogenic response and exhibit extended half-lives *in vivo* in comparison to peptides that are not PEGylated when administered *in vivo*. Methods for PEGylating proteins and peptides are

known in the art (see, for example, U.S. Patent No. 4,179,337). The subject peptidomimetics can also be modified to improve cell membrane permeability. In one embodiment, cell membrane permeability can be improved by attaching a lipophilic moiety, such as a steroid, to the peptidomimetic. Other groups known in the art can be linked to peptidomimetics of the present invention.

The subject invention also concerns a packaged dosage formulation comprising in one or more containers at least one peptidomimetic of the subject invention formulated in a pharmaceutically acceptable dosage. The package can contain discrete quantities of the dosage formulation, such as tablet, capsules, lozenge, and powders. The quantity of peptidomimetic in a dosage formulation and that can be administered to a patient can vary from about 1 mg to about 2000 mg, more typically about 1 mg to about 500 mg, or about 5 mg to about 250 mg, or about 10 mg to about 100 mg.

Materials and Methods

Cells and reagents. Src-transformed NIH3T3/v-Src, NIH3T3/v-Src/pLucTKS3, NIH3T3/v-Src/pRLSRE, and Ras-transformed NIH3T3/v-Ras fibroblasts, human breast carcinoma MDA-MB-231, MDA-MB-435, MDA-MB-453 and MDA-MB-468 cells, as well as human lung carcinoma A459 cells have been previously described (Turkson *et al.* (2001); Yamauchi *et al.* (1993)). Cells were grown in Dulbecco's modified Eagle's medium (DMEM) containing 5% iron-supplemented bovine calf serum (BCS), with or without G418 or zeocin. The Apo-BrdU kit was from PharMingen (San Diego, CA).

Peptides. Peptides used in studies include PY*LKTK-AAVLLPVLLAAP (SEQ ID NO. 1) and PYLKTK-AAVLLPVLLAAP (SEQ ID NO. 2) (the underlined amino acid sequence represents the membrane translocating sequence (MTS) (Rojas, M. *et al.* (1998))) and peptidomimetics based on PY*L and AY*L (where Y* = phosphotyrosine). Peptides were synthesized by the Peptide Synthesis Laboratory, Queen's University, Kingston, ON, Canada. Peptidomimetics were synthesized manually using standard Fmoc solid phase chemistry. Peptides or peptidomimetics were used at concentrations up to 1 mM as indicated.

Plasmids. The Stat3 reporter, pLucTKS3, driving expression of the firefly luciferase gene and the Stat3-independent plasmid, pRLSRE, containing two copies of the serum response

element (SRE) from the *c-fos* promoter (Turkson, J. *et al.* (1998); Zhang, Y. *et al.* (2000)) that drives renilla luciferase gene (Promega; Madison, WI) expression, have been previously described (Turkson, J. *et al.* (2001); Turkson, J. *et al.* (1999)).

Recombinant baculoviruses and infection of Sf-9 insect cells. Stat1, Stat3, Jak1 and c-

Src recombinant baculoviruses and infection of Sf-9 insect cells have been previously described (Zhang, Y. *et al.* (2000)). For protein expression of activated Stat1 or Stat3, Sf-9 insect cells were infected with viruses expressing either Stat1 or Stat3 in combination with viruses expressing Jak1 and/or c-Src.

Cytosolic extract preparation and luciferase assays. Cytosolic extract preparation from fibroblasts and luciferase assays were previously described (Turkson, J. *et al.* (1998); Turkson, J. *et al.* (1999)). Briefly, after two washes with PBS and equilibration for 5 min with 0.5 ml PBS-0.5 mM EDTA, cells were scraped off the dishes and the cell pellet was obtained by centrifugation (4,500 X g, 2 min, 4 °C). Cells were resuspended in 0.4 ml of low-salt HEPES buffer (10 mM HEPES (pH 7.8), 10 mM KCl, 0.1 mM EGTA, 0.1 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, and 1 mM dithiothreitol) for 15 min, lysed by the addition of 20 µl of 10% Nonidet P-40 (NP-40), and centrifuged (10,000 X g, 30 s, 4 °C) to obtain the cytosolic supernatant, which was used for luciferase assays (Promega) measured with a luminometer. Cytosolic lysates containing activated Stat3 or Stat1 that were used for dissociation-reassociation analysis were prepared from baculovirus-infected Sf-9 insect cells as previously described (Turkson, J. *et al.* (2001); Zhang, Y. *et al.* (2000)). Briefly, cultured dishes of Sf-9 cells were washed twice with ice-cold 1X PBS and then PBS containing 1 mM sodium orthovanadate. Cells were then lysed in 1% NP-40 lysis buffer (50 mM HEPES (pH 7.9), 150 mM NaCl, 1% NP-40, 20 mM NaF, 1 mM sodium orthovanadate, 1 mM tetrasodium pyrophosphate, 1 mM dithiothreitol, 0.5 mM phenylmethylsulfonyl fluoride, 2 mM EGTA, 2 mM EDTA, 0.1 µM aprotinin, 1 µM leupeptin, and 1 µM antipain) on ice for 10 min, and centrifuged (13,000 X g, 30 s, 4 °C) to obtain lysate.

Nuclear extract preparation and gel shift assays. Nuclear extracts were prepared from cell lines and used for electrophoretic mobility shift assay (EMSA) as previously described (Garcia, R. *et al.* (1997); Yu, C.L. *et al.* (1995); Turkson, J. *et al.* (1998)). In some cases, cells were pre-treated with peptidomimetics for the indicated times (12-48 h) prior to harvesting for

nuclear extract preparation. In other studies, nuclear extracts were pre-incubated with peptidomimetics for 30 min at room temperature prior to incubation with radiolabeled probe. The ³²P-radiolabeled oligonucleotide probes used are hSIE (high affinity *sis*-inducible element, m67 variant, 5'-AGCTTCATTTCCTGTAATCCCTA-3') (SEQ ID NO. 3) that binds both Stat1 and Stat3 (Garcia, R. *et al.* (1997); Wagner B.J. *et al.* (1990)) and MGFe (mammary gland factor element from the bovine β -casein gene promoter, 5'-AGATTCTAGGAATTCAA-3') (SEQ ID NO. 4) that binds Stat1 and Stat5 (Gouilleux, F. *et al.* (1995); Seidel, H.M. *et al.* (1995)).

Dissociation-reassociation analysis. Two independent preparations of lysate (from baculovirus-infected Sf-9 cells) containing either active Stat1:Stat1 or Stat3:Stat3 were pooled together. Aliquots of mixed lysates of equal total protein were pre-treated with or without 30-300 μ M PY*LKTK (SEQ ID NO. 5) or 3-1000 μ M ISS 610 for 30 min prior to incubation with ³²P-labeled hSIE and subjected to EMSA (Shuai, J.I *et al.* (1994)).

Cell proliferation, soft-agar growth and Apo-BrdU labeling studies. Proliferating fibroblasts and human tumor cells were counted by phase-contrast microscopy for viable cells (using tyrpan blue exclusion). Colony formation assays in six-well dishes and quantification of colonies by iodonitrotetrazolium violet have been previously described (Turkson, J. *et al.* (1999)). Treatment of cells with inhibitors was initiated 1 day after seeding cells by adding 75 μ l of medium with or without inhibitor, and repeated every two to three days. Apoptosis was measured by Apo-BrdU labeling and following the supplier's (PharMingen; San Diego, CA) instructions. Cells (NIH3T3 or NIH3T3/v-Src) were first treated with or without PY*LKTK-MTS (SEQ ID NO. 1), or ISS 610 for 48 h prior to labeling and than analyzed by flow cytometry for detection of apoptotic cells.

All patents, patent applications, provisional applications, and publications referred to or cited herein are incorporated by reference in their entirety, including all figures and tables, to the extent they are not inconsistent with the explicit teachings of this specification.

Following are examples which illustrate procedures for practicing the invention. These examples should not be construed as limiting. All percentages are by weight and all solvent mixture proportions are by volume unless otherwise noted.

Example 1—Design of PY*L peptidomimetics: substitutions of proline with aromatic groups generate strong disruptors of Stat3 DNA-binding activity *in vitro*

It has been reported that PY*LKTK (SEQ ID NO. 5), the putative Stat3 SH2 domain-binding sequence, and the tripeptides PY*L and AY*L, disrupt Stat3:Stat3 dimer formation and subsequent Stat3 DNA-binding activity. Peptidomimetics of the invention can be prepared by replacing prolyl or alanyl residue at the peptide bond that is N-terminal to the phosphotyrosine (Y*) of a peptide such as PY*L or AY*L with an organic substituent such as an optionally substituted aryl or heteroaryl group. The activities of STATs were measured in nuclear extracts (prepared from epidermal growth factor (EGF)-stimulated fibroblasts) as DNA-bound protein complexes by electrophoretic mobility shift assay (EMSA). STAT-DNA complexes detected include Stat3:Stat3 homodimers (upper band), Stat1:Stat3 heterodimers (intermediate band) and Stat1:Stat1 homodimers (lower band) (Figure 1A) when a ³²P-labeled hSIE oligonucleotide probe is used, or Stat5:Stat5 (upper band) and Stat1:Stat1 dimers (lower band) (Figure 1B) when ³²P-labeled MGFe is used as a probe.

Pre-incubation of peptidomimetics of the invention with nuclear extracts results in potent and dose-dependent inhibition of DNA-binding activities of Stat3, and to a lesser extent of Stat1 or Stat5 (Figures 1A and 1B). From densitometric analysis of band intensities, the IC₅₀ values (concentration of peptidomimetic at which DNA-binding activity is reduced by 50%), which are reported in Table 3, were determined. Peptidomimetics ISS 610 and ISS 637 exhibited a 5-fold increase in potency (compared to tripeptides PY*L or AY*L) in inhibiting Stat3 DNA-binding activity *in vitro* (IC₅₀ value decreased from 217 ± 55 μM (AY*L) or 182 ± 15 μM (PY*L) to 42 ± 23 μM (ISS 610) or 55 ± 35 μM (ISS 637)) (Table 3 and Figure 1A). In their inhibitory activities, these peptidomimetics also show preference for Stat3 over Stat1 or Stat5. The non-tyrosine-phosphorylated form of ISS 610 (ISS 610NP) has no effect on Stat3 (and Stat1 or Stat5) DNA-binding activity *in vitro* (Figures 1A and 1B, bottom panels), reflecting the importance of the pTyr for disruption of Stat3 by peptidomimetics of the invention. These findings provide support for the engagement of pTyr-SH2 interaction as being the basis for Stat3 dimer disruption by peptidomimetics of the invention, such as ISS 610 (see below).

Peptidomimetics ISS 493, ISS 610, and ISS 637 exhibited preferential suppression of Stat3 activity over those of Stat1 or Stat5 (Table 4), while others, such as in ISS 593, have

enhanced potency against Stat5 over Stat3 (IC_{50} values of $10 \pm 6 \mu M$). The inhibition of Stat5 has important implications for some types of human tumors, such as chronic myelogenous leukemia, which depend on constitutively-active Stat5 for growth and survival (Bowman, T. *et al.* (2000); Smithgall, T.E. *et al.* (2000); Lin, J. *et al.* (2000)).

5

Example 2—Disruption of STAT dimerization: evidence for dissociation of STAT dimers

A STAT dimerization disruption model (Turkson, J. *et al.* (2001)) has been proposed to define the interaction of Stat3:Stat3 dimers with PY*LKTK (SEQ ID NO. 5), PY*L, and AY*L. In this model, phosphopeptides engage in pTyr-SH2 interactions with STAT:STAT dimers, which results in STAT dimer dissociation into monomers (in complexes with phosphopeptides), some of which may in turn reassociate into STAT dimers. Using this model, dissociation-reassociation analysis (Shuai, K. *et al.* (1994)) was performed with two independent cell lysate preparations, one containing only active Stat1:Stat1 dimers and the other only Stat3:Stat3 dimers. Lysates were mixed together with or without PY*LKTK (SEQ ID NO. 5) or peptidomimetic ISS 610 or its non-phosphorylated counterpart, ISS 610NP, and then incubated with radiolabeled hSIE probe (SEQ ID NO. 3) and subjected to EMSA analysis.

Either cell lysate preparation alone shows one band corresponding to Stat1:Stat1 dimers (Figures 2A and 2B, lane 1) or Stat3:Stat3 dimers (Figures 2A and 2B, lane 2). For mixed cell lysates, two bands with migrations consistent with Stat1:Stat1 dimers (lower band) or Stat3:Stat3 dimers (upper band) (Figures 2A and 2B, lane 3) are observed in the absence of the phosphopeptide, PY*LKTK (SEQ ID NO. 5), or ISS 610. However, EMSA analysis of mixed cell lysates that are pre-incubated with PY*LKTK (SEQ ID NO. 5) or ISS 610 shows three bands: i) lower and upper bands corresponding to Stat1:Stat1 and Stat3:Stat3 dimers, respectively (Figures 2A and 2B, lanes 4 to 8), which are of decreasing intensities with increasing concentrations of phosphopeptide or peptidomimetic, consistent with results in Figure 1A; and ii) appearance of an additional intermediate band representing Stat1:Stat3 heterodimers (Figures 2A and 2B, lanes 4 to 7) (Shuai, K. *et al.* (1994)) that hitherto were not present and could only have formed from random reassociation of two dissociated phosphorylated monomers. Results also show decreasing band intensities or complete disappearance of the three STAT-DNA complexes at higher concentrations of PY*LKTK (SEQ ID NO. 5) or ISS 610

(Figure 2A, lane 10, and Figure 2B, lane 8) due primarily to total disruption of all STAT:STAT dimers and formation of only complexes of STAT protein with peptidomimetics (or phosphopeptides). The apparently stronger disruption of Stat1:Stat1 dimer might be due to a relatively lower amount of Stat1 protein in starting material (lysate) compared to Stat3 protein.

5 The non-phosphorylated ISS 610NP has no effect (data not shown), which demonstrates the importance of pTyr in peptidomimetics of the invention for disruption of Stat3 dimers.

Example 3—Peptidomimetic selectively blocks Stat3 signaling and constitutively-active Stat3-dependent v-Src transformation

10 PY*LKTK-MTS (SEQ ID NO. 1) (MTS, membrane translocation sequence, is a sequence of hydrophobic amino acids that facilitates transport of peptides across cell membranes) (Rojas, M. *et al.* (1998)) inhibits constitutive Stat3 activation in Src-transformed fibroblasts (Turkson J. *et al.* (2001)). Peptidomimetics which are phosphorylated on tyrosine have reduced membrane-permeability, particularly if they do not contain MTS to enhance

15 permeability. Thus, peptidomimetic ISS 610 was used at 1 mM concentrations in whole-cell studies. Luciferase reporter assays on Src-transformed mouse-cells that stably express Stat3-dependent and Stat3-independent dual luciferase reporters (Turkson J. *et al.* (2001)), and are treated with ISS 610 show a significant suppression of transcriptional induction of the Stat3-dependent luciferase reporter, pLucTKS3, with no effect on the induction of the Stat3-

20 independent luciferase reporter, pRLSRE (Figure 3A). Similarly, DNA-binding studies and EMSA analysis reveal a time-dependent reduction of Stat3 activation in Src-transformed fibroblasts (Figure 3C), human non-small cell lung carcinoma, (A549) and breast carcinomas (MDA-MB-231, MDA-MB-435, and MDA-MB-468) cells that harbor constitutive Stat3 activation (Garcia R. *et al.* (2001); Song, L. *et al.* (2003)) and are treated with ISS 610 (Figure

25 3E, second lane for each cell line). Inhibition of Stat3-dependent luciferase induction and Stat3 activation in whole cells was not complete at 48 h post-treatment (Figure 3A and 3C), possibly due to low intracellular levels of peptidomimetic as a result of weak uptake, rapid degradation, or both. In other studies, the effect of ISS 610 on ligand (EGF)-induced STAT activation in mouse fibroblasts was evaluated and preferential inhibition of activation of Stat3, and to a lesser extent

30 of that of Stat1 was observed (data not shown). Moreover, the non-phosphorylated ISS 610NP

has no effect on Stat3 activation in Src-transformed cells or Stat3-mediated gene expression (Figures 3B and 3D), indicating that pTyr is required for disruption of interaction between Stat3 dimers. Thus, peptidomimetics of the invention can selectively suppress constitutive Stat3 signaling in whole cells.

5 Previous studies with PY*LKTK-MTS (SEQ ID NO. 1) (Turkson, J. *et al.*, 2001)) confirmed that inhibition of constitutive Stat3 activation blocks Src transformation (Turkson, J. *et al.* (1998); Turkson, J. *et al.* (1999); Bromberg, J.R. *et al.* (1998)). Using growth in soft agar as a measure of transformation, the effect of representative peptidomimetic ISS 610 was determined. Growth of NIH3T3/v-Src in soft-agar suspension is significantly suppressed by the
10 addition of ISS 610 (Figure 3F). In contrast, ISS 610 has no effect on soft-agar growth of Stat3-independent Ras-transformed fibroblasts (NIH3T3/v-Ras) (Figure 3F). These findings indicate that selective blocking of constitutive Stat3 signaling by the peptidomimetic inhibitor ISS 610 suppresses transformation of mouse fibroblasts by v-Src.

15 Example 4—ISS 610 induces growth inhibition of malignant cells that contain persistent Stat3 activity

Stat3 has an essential role in cell proliferation, and constitutively-active Stat3 is required for growth of transformed and tumor cells that harbor aberrant Stat3 signaling. Studies were performed to determine the effects of the peptidomimetic-mediated inhibition of Stat3 on cell
20 proliferation using trypan blue exclusion for viable cell counts. Results show that treatment with ISS 610 (or PY*LKTK-MTS for comparison) (SEQ ID NO. 1) of Src-transformed mouse fibroblasts (NIH3T3/v-Src), or the human breast carcinoma cells MDA-MB-231 and MDA-MB-435 that harbor constitutively-active Stat3 significantly suppresses proliferation compared to control (non-treated cells) (Figures 4B and 4C). In comparison to the effect observed for
25 PY*LKTK-MTS (SEQ ID NO. 1), the inhibition of cell proliferation by ISS 610 was only partial. Unlike the phosphopeptide, which is linked at the C-terminus to the membrane-translocation sequence (MTS) (Rojas, M. *et al.* (1998)) to facilitate intracellular uptake across cell membrane, ISS 610 lacks this MTS and, therefore, may not be efficiently taken up into cells, which will in turn be reflected in its biological activity. Treatment of cells that lack constitutive
30 Stat3 activity (*e.g.*, normal NIH3T3 cells, Ras-transformed counterparts (NIH3T3/v-Ras), or

MDA-MB-453 cells) shows no effect on cell proliferation (Figure 4A, data not shown). Thus, ISS 610 does not appear to have any general cytostatic or cytotoxic effects. These findings show that peptidomimetics of the invention inhibit growth of transformed mouse and human tumor cells that harbor constitutive Stat3 activity.

5

Example 5—Peptidomimetics induces apoptosis in transformed fibroblasts that exhibit persistent Stat3 activity

Because one of the functions of Stat3 is to protect transformed or tumor cells from apoptosis (Catlett-Falcone, R. *et al.* (1999b); Epling-Burnette, P.K. *et al.* (2001); Grandis, J.R. *et al.* (2000); Bowman, T. *et al.* (2000b); Bromberg, J.F. *et al.* (1999)), the ability of peptidomimetics of the invention (and PY*LKTK-MTS for comparison) (SEQ ID NO. 1) to induce apoptosis in Stat3-dependent transformed fibroblasts was evaluated. Viral Src-transformed NIH3T3/v-Src fibroblasts were treated with or without compounds for 48 h. Treated cells were then labeled with Apo-BrdU (PharMingen; San Diego, CA) for detection of DNA breaks. Results from flow cytometric analysis of cells show dramatic increases in incorporated BrdUTP in Src-transformed fibroblasts treated with PY*LKTK-MTS (SEQ ID NO. 1) or ISS 610 compared to controls (non-treated cells or treated with control peptide, PYLKTK-MTS (SEQ ID NO. 2)) or to normal NIH3T3 fibroblasts treated with peptides or peptidomimetic (Table 5). Together, these findings show that PY*LKTK (SEQ ID NO. 5) and ISS 610 induce apoptosis in Stat3-dependent transformed cells but not in normal cells. This observation reflects the inhibition of Stat3 activity and its biological function, which together with the other data demonstrates that selective suppression of constitutive Stat3 activation and its biological function induces apoptosis in model transformed cells that harbor constitutive Stat3 activity.

25

Table 3. Disruption of Stat3 DNA-binding activity by peptidomimetics having the structure shown in formula I and R group shown.

Nuclear extracts containing active Stat3 were pre-incubated for 30 min with or without peptidomimetics prior to incubation with radiolabeled hSIE probe and analysis by EMSA.

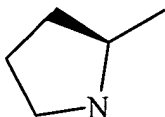
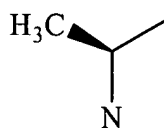
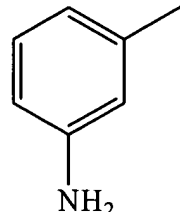
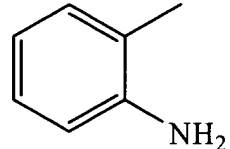
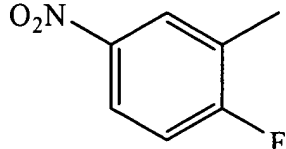
Compound or Compound Designation	R	IC ₅₀ (μM)**
Prolylphosphotyrosylleucine		182 +/- 15
Alanylphosphotyrosylleucine		217 +/- 55
ISS 248		ne
ISS 265		ne
ISS 375		ne

Table 3. Disruption of Stat3 DNA-binding activity by peptidomimetics having the structure shown in formula I and R group shown.

Nuclear extracts containing active Stat3 were pre-incubated for 30 min with or without peptidomimetics prior to incubation with radiolabeled hSIE probe and analysis by EMSA.

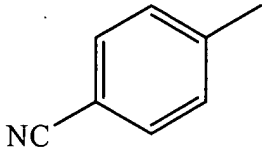
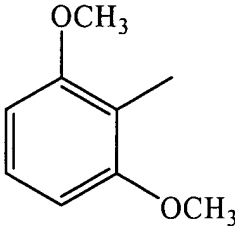
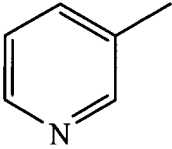
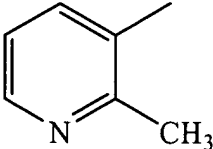
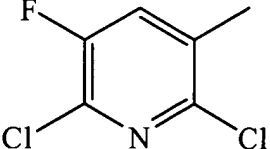
Compound or Compound Designation	R	IC ₅₀ (μM)**
ISS 610		42 +/- 23
ISS 637		55 +/- 35
ISS 219		232 +/- 16
ISS 221		75 +/- 36
ISS 593		48 +/- 32

Table 3. Disruption of Stat3 DNA-binding activity by peptidomimetics having the structure shown in formula I and R group shown.

Nuclear extracts containing active Stat3 were pre-incubated for 30 min with or without peptidomimetics prior to incubation with radiolabeled hSIE probe and analysis by EMSA.

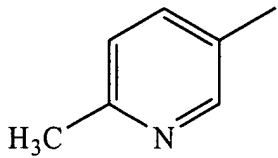
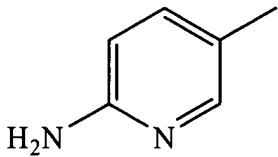
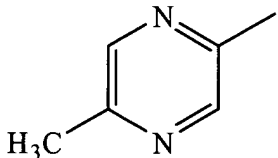
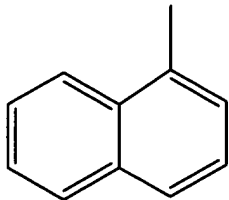
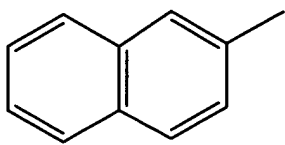
Compound or Compound Designation	R	IC ₅₀ (μM)**
ISS 223		225 +/- 15
ISS 249		ne
ISS 493		38 +/- 16
ISS 352		410 +/- 15
ISS 353		650 +/- 22

Table 3. Disruption of Stat3 DNA-binding activity by peptidomimetics having the structure shown in formula I and R group shown.

Nuclear extracts containing active Stat3 were pre-incubated for 30 min with or without peptidomimetics prior to incubation with radiolabeled hSIE probe and analysis by EMSA.

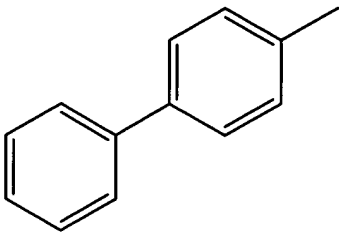
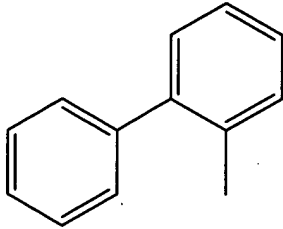
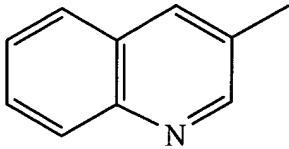
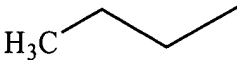
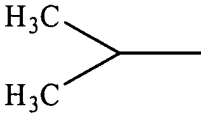
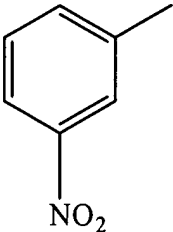
Compound or Compound Designation	R	IC ₅₀ (μM)**
ISS 355		ne
ISS 360		420 +/- 35
ISS 363		643 +/- 43
ISS230	H ₃ C—	ne
ISS231		ne

Table 3. Disruption of Stat3 DNA-binding activity by peptidomimetics having the structure shown in formula I and R group shown. Nuclear extracts containing active Stat3 were pre-incubated for 30 min with or without peptidomimetics prior to incubation with radiolabeled hSIE probe and analysis by EMSA.		
Compound or Compound Designation	R	IC ₅₀ (μM)**
ISS234		ne
ISS227		245 +/- 6

Values are the means and standard deviations of at least 3 independent assays. **IC₅₀, concentration of peptidomimetic at which DNA-binding is reduced by 50%; ne, no effect at 1 mM. Results are representative peptidomimetics from over 80 that have been evaluated.

Table 4. Selective disruption of STAT family members by peptidomimetics

Nuclear extracts containing active Stat1, Stat3 and Stat5 were pre-incubated with or without peptidomimetics for 30 min prior to incubation with radiolabeled hSIE probe (SEQ ID NO. 3) and EMSA analysis.

<i>IC</i> ₅₀ values (μM) against STAT Dimers**				
Peptidomimetic	Stat3:Stat3	Stat1:Stat3	Stat1:Stat1	Stat5:Stat5
ISS 221	75+/-36	455+/-97	310+/-74	50+/-12
ISS 493	38+/-16	230+/-22	273+/-22	300+/-22
ISS 593	48+/-32	87+/-15	175+/-65	10+/-6
ISS 610	42+/-23	125+/-15	310+/-145	285+/-32
ISS 637	55+/-35	195+/-12	255+/-55	695+/-123

**Values are the means and standard deviations of at least 3 independent assays. *IC*₅₀, concentration of peptidomimetic at which DNA-binding is reduced by 50%; ne, no effect at 1 mM. Results are representative peptidomimetics from over 80 that have been evaluated.

Table 5. Flow cytometric analysis for percent of apoptotic cells treated with PY*LKTK-MTS (SEQ ID NO. 1) and ISS 610.

	NIH3T3	NIH3T3/v-Src
Control	2.1%	1.8%
1 mM PYLKTK-MTS (SEQ ID NO. 2)	1.2%	0.4%
1 mM PY*LKTK-MTS (SEQ ID NO. 1)	3.7%	27.6%
1 mM ISS 610	0.3%	21.4%

Cells were treated with compounds for 48h, labeled with Apo-BrdU and analyzed by flow cytometry for percent apoptotic cells. Control represents no treatment.

10 It should be understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application.

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